Clinical Proteomic Technology Assessment for Cancer

Sample Collection and Biospecimens

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And

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Biospecimens Are Key to the Future of Molecular Medicine

- **Biorepositories with high-quality biospecimens and data are needed to:**
  - **Support development of genomic, proteomic analysis**
  - **Identify targets for therapy, detection, and prevention**
  - **Develop a molecular-based taxonomy of cancer**
  - **Integrate huge amounts of molecular & clinical data**
  - **Ultimately realize the era of personalized medicine**

Biospecimen Issues for the NCI and Cancer Research Community

- There are no national biorepository standards
- NCI's current systems do not enable accurate accounting or analysis of funded biospecimen resources
- NCI does not have guidance for biospecimen quality control, ethical and legal policies, access or retention of specimens
- Given these issues - data supporting certain areas of science are in doubt (we don't know the impact of many variables on cancer and normal tissues)

Recent NCI Activities

- 2005/2006:
  - Biorepository Coordinating Committee (BCC) formed
  - National Biospecimen Network Prostate SPORE Pilot launched
  - Biospecimen whitepapers finalized from background documents
  - Two workshops held – biorepository operations & ethical/legal/policy issues
  - **NCI Office of Biorepositories and Biospecimen Research (OBBR) established –** Dr. Carolyn Compton, Director
  - First generation NCI guidelines for biorepositories being finalized

NCI Biorepository Coordinating Committee (BCC)

- Membership: Representatives from NCI divisions assembled to address harmonization of policies for biorepositories across the NCI
- Mission: To define quality Best Practices for NCI-supported biorepositories
- Process: Exhaustive review of the literature from authoritative sources and extensive input from the scientific community. Two wide-ranging workshops in summer 2005.
- Result: Development of 1st generation guidelines for NCI biorepositories

NCI 1st Generation Biorepository Guidelines Overview

NCI is developing biorepository guidelines that include recommendations for:

- **Common best practices for research biorepositories**
- **Quality assurance and quality control programs**
- **Implementation of informatics systems**
- **Addressing ethical, legal, and policy issues**
- **Establishing reporting mechanisms**
- **Providing administration and management structure**

Specimen Collection, Processing, Storage, Retrieval, and Dissemination

Biorepositories should assure that:

1. **Specimen handling is appropriate to the specimen type and for the intended study.**
2. **Written SOPs document all protocols and all laboratory functions.**
3. **Specimens are fully annotated with key collection, processing, and storage data.**
4. **A tracking system monitors specimen inventory.**
5. **A comprehensive quality management system is in place.**
6. **Biorepository personnel are trained to adhere to SOPs.**
7. **A pathologist directs the collection and processing of surgical and autopsy specimens.**
8. **Specimens are stored in a stabilized state without unnecessary thawing/refreezing.**
9. **Specimen disposal is performed according to clear rules.**

Specimen Collection, Processing, Storage, Retrieval, and Dissemination (continued)

10. Collection and processing time are minimized and documented as appropriate for the specimen type.
11. Sample retrieval is designed to minimize disruption of the stable environment.
12. Storage equipment performance is reviewed and recorded regularly.
13. Specimen containers will not interfere with analytical goals.
14. Specmen-appropriate biosafety, packaging, and shipping procedures are followed.
15. Specimen retrieval procedures safeguard specimen quality.
16. Shipping temperature is controlled and monitored as necessary.
17. Material Transfer Agreements are used.
18. Personnel are trained to adhere to specimen shipping regulations.

Quality Assurance, Quality Control

1. A quality management system describing quality assurance and quality control procedures is in place.
2. The biorepository maintains QA/QC training records for personnel.
3. The biorepository adheres to and periodically reviews SOPs.
4. Security systems, including alarms and backup power, are in place.
5. Data management systems include a computerized inventory tracking system.
6. A facility disaster plan is in place.
7. All equipment is maintained properly according to SOPs.

Legal & Ethical Issues
Informed Consent

The collection of tissue for research requires the informed consent of the tissue donor. The consent document must contain the following elements (adapted from CFR Title 45, Part 46):

- **A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;**
- **A description of any reasonably foreseeable risks or discomforts to the subject;**
- **A description of any benefits to the subject or to others;**
- **A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;**
- **A statement describing the extent to which confidentiality of records identifying the subject will be maintained;**

Informed Consent – continued

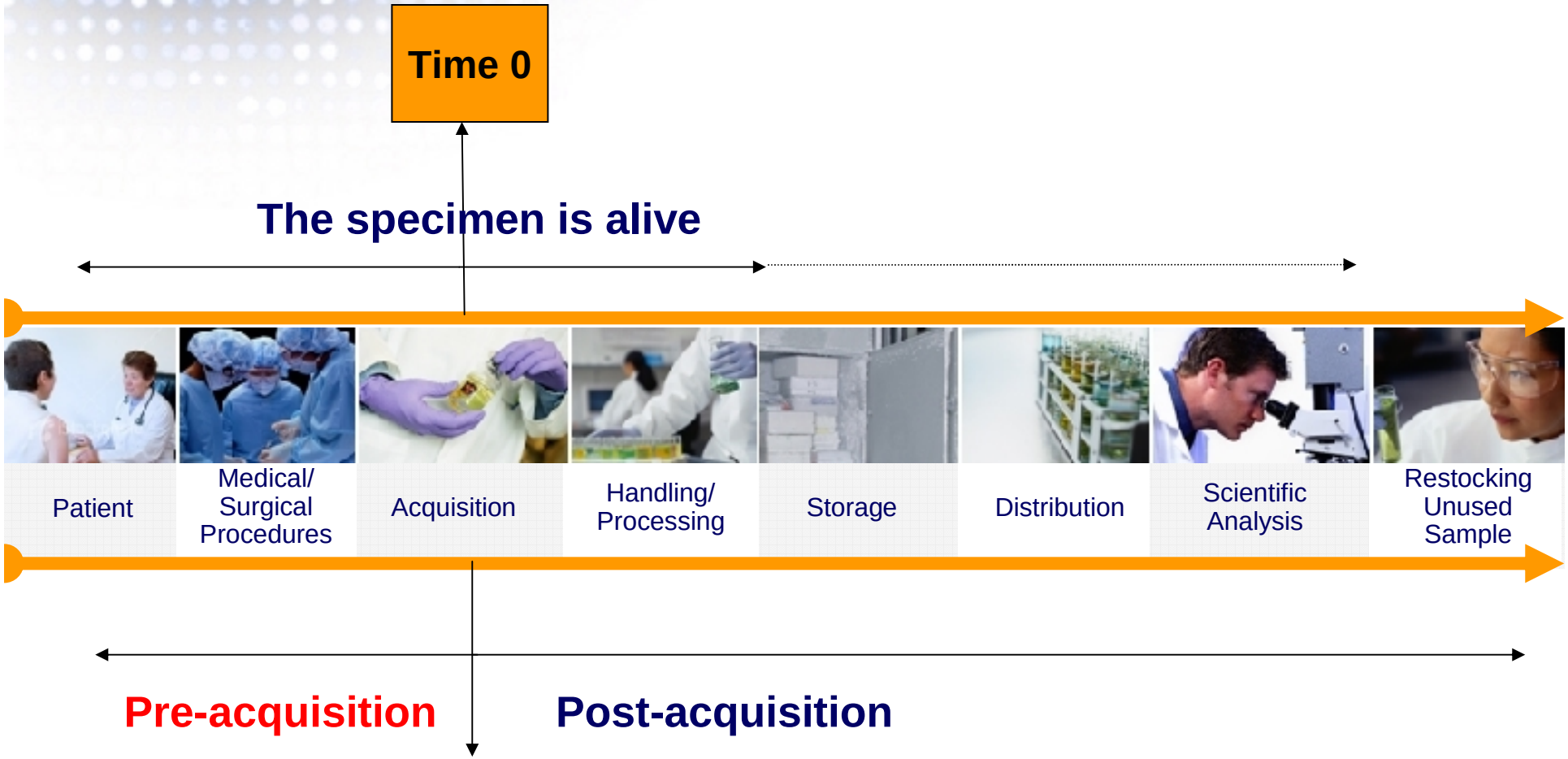
- For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained;
- An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and
- A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Do the 1st Generation Guidelines Impact This Project?

- Basic specimen collection, processing and storage guidelines need to be followed, as appropriate for the specimen type.
- A quality assurance/quality control plan should be in place.
- Specimens must be collected using proper informed consent and following appropriate regulations.
- Other portions of the guidelines should be reviewed, e.g. biosafety, informatics, data collection

For the full set of guidelines see:
http://biospecimens.cancer.gov

Lifecycle of a Biospecimen: Biospecimen Research



Time between tissue removal and paraffin embedding

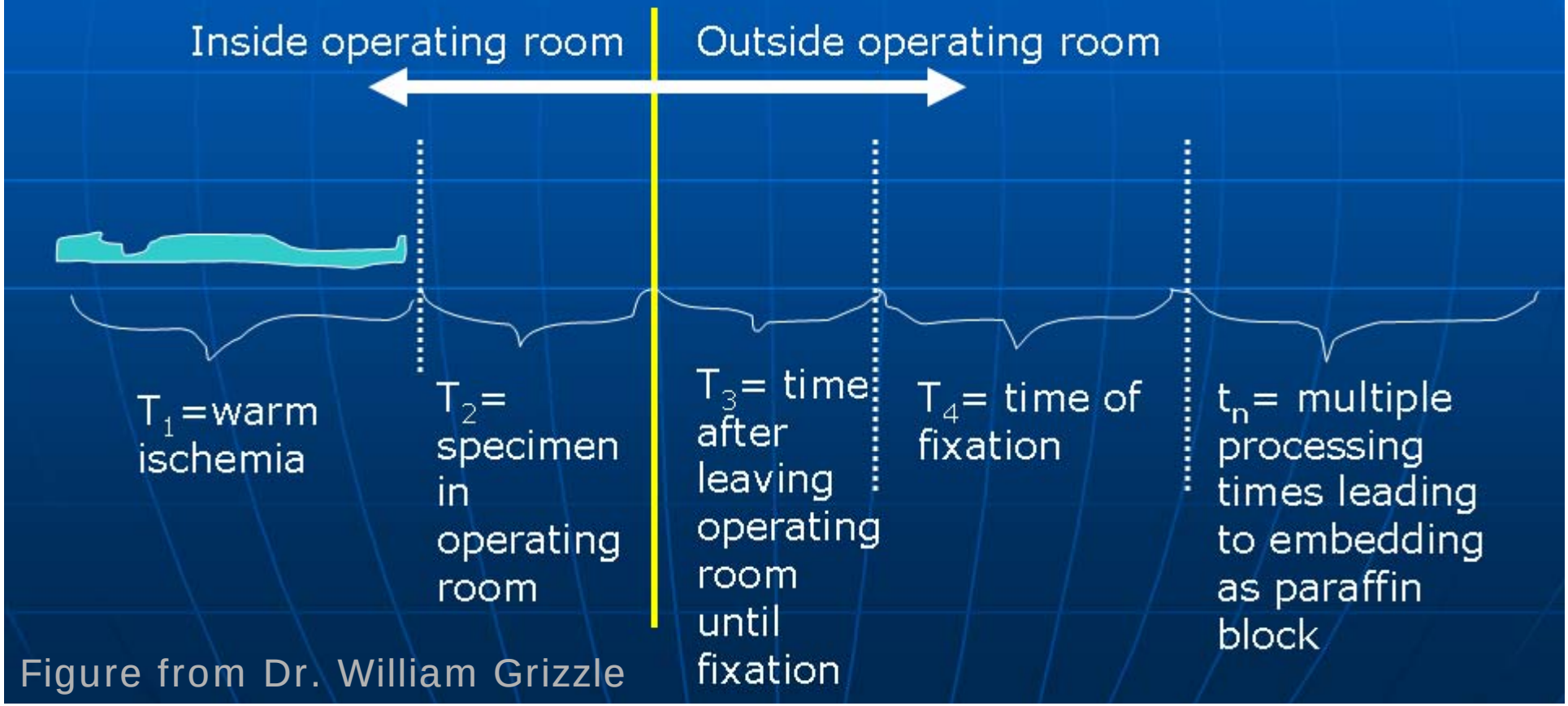


Figure from Dr. William Grizzle

Lifecycle of a Biospecimen: Biospecimen Research

Post-acquisition variables:

- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots
- Storage temperature
- Storage duration
- Storage in vacuum

Pre-acquisition variables:

- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time
- Blood pressure variations
- Intra-op blood loss
- Intra-op blood administration
- Intra-op fluid administration

Factors affecting specimen stability

- Additives used in blood collection may not be appropriate for some analyses
  - Prime anticoagulants - Heparin, EDTA, Acid-citrate-dextrose
  - EDTA – prevents some protease activity
  - Or no additives for serum collection
- Time elapsed between specimen collection and processing/stabilization
- Sterility of instruments, surfaces and equipment
- Enzymatic degradation, especially of RNA and protein
- Repeated thaw-refreeze cycles

From ISBER Best Practices

Cell Preservation Technology 3: 5-48, 2005.

Key Issues For Human Biospecimen Collection and Protein Analysis

Protein degradation after corporal extraction:

Protein stability is dependent on the specific protein and presence of proteases, phosphatases etc.

- **Keratins stable for hours**
- **Phosphoproteins stable for minutes**

Other factors:

- **Length of Time until fixation or freezing**
- **Temperature of biospecimen during procurement**
- **Tissue type**
- **Ischemic time**

Courtesy K. Calvo NCI

Stability of phosphoprotein as a biological marker of tumor signaling.

Baker AF, Dragovich T, Ihle NT, Williams R, Fenoglio-Preiser C, Powis G.

Clinical Cancer Research. 2005 Jun 15;11(12):4338-40.

PURPOSE: The purpose of the study was to evaluate the stability of phosphoprotein as a marker of signaling activity in human tumors using clinical samples and xenografts. EXPERIMENTAL DESIGN: The expression of phospho-Ser473-Akt (p-Akt) was assessed by immunohistochemistry in paraffin-embedded samples from patients enrolled in a Southwest Oncology Group clinical trial of gastroesophageal junction tumors and by immunohistochemistry and Western blotting in human colon tumor xenografts at various times after removal from the animal. **RESULTS: Clinical samples had evaluable p-Akt staining only when obtained as biopsies (9 of 13) and no staining was observed in tumors obtained as surgically resected samples (0 of 15). In HT-29 colon cancer xenografts, p-Akt staining was present in fresh sample but not in tissue that had been allowed to stand for 30 minutes at room temperature.** Western blotting of HT-29 tumor xenografts at room temperature showed a slow decrease in **total Akt with a half-life of 180 minutes and a rapid decrease in p-Akt with a half-life of 20 minutes.** CONCLUSIONS: Caution should be used when using phosphoprotein levels in human tumor specimens to measure intrinsic signaling activity or drug effects because of the potential for rapid dephosphorylation. **Rapid processing of biopsies is essential and postoperative surgical samples may be of limited value because of the time to fixation.**

Key Issues For Human Biospecimen Collection and Protein Analysis

Method of freezing or fixing and effect on protein yield

A. Fresh frozen tissue gives the highest protein yield

- Snap freezing in Liquid Nitrogen
- Embedding in OCT in a cryomold with subsequent freezing
- Storage temperature: -80^{o} C freezer,
  -150 vapor phase nitrogen. -196 liquid nitrogen

B. Ethanol fixed tissue:

- Protein yield can be as high as 70% of fresh frozen
- Paraffin embedding, ease of sectioning, storage at RT
- Compatible with IHC, and morphologic dx

C. Formalin Fixation:

- Crosslinks proteins, generally low protein yield
- Excellent morphology for histopathologic diagnosis
- Compatible with immunohistochemistry

Courtesy K. Calvo NCI

Liquid Nitrogen Freezers

May offer longer stability

for certain proteins

Dry shippers – LN temp for weeks

Analytical Techniques Compatible with Direct Analysis of Proteins in Clinically Derived Human Biospecimens

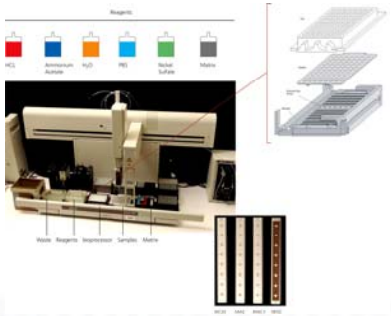
Mass Spectrometry Based Platforms

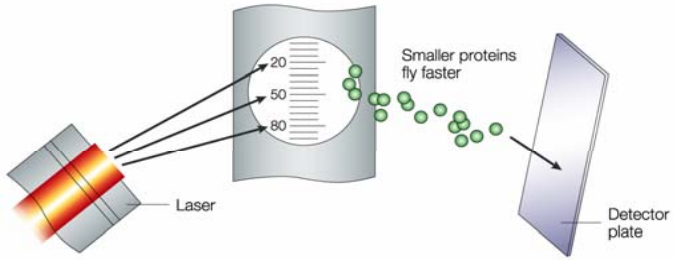
Holds much promise for:

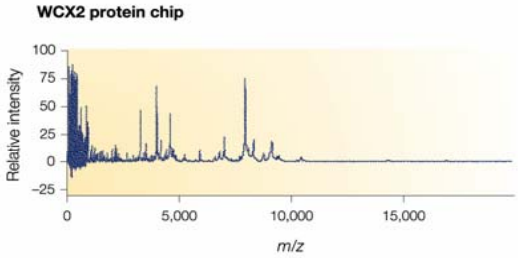
- **Biomarker discovery**
- **Serum and tissue based- proteomic pattern diagnostics**

Research tool, need for standardization, validation

Advantages: **Small amount of serum (1 microL) or tissue required**
High throughput, hundreds + proteins analyzed
Sensitive for low abundance proteins







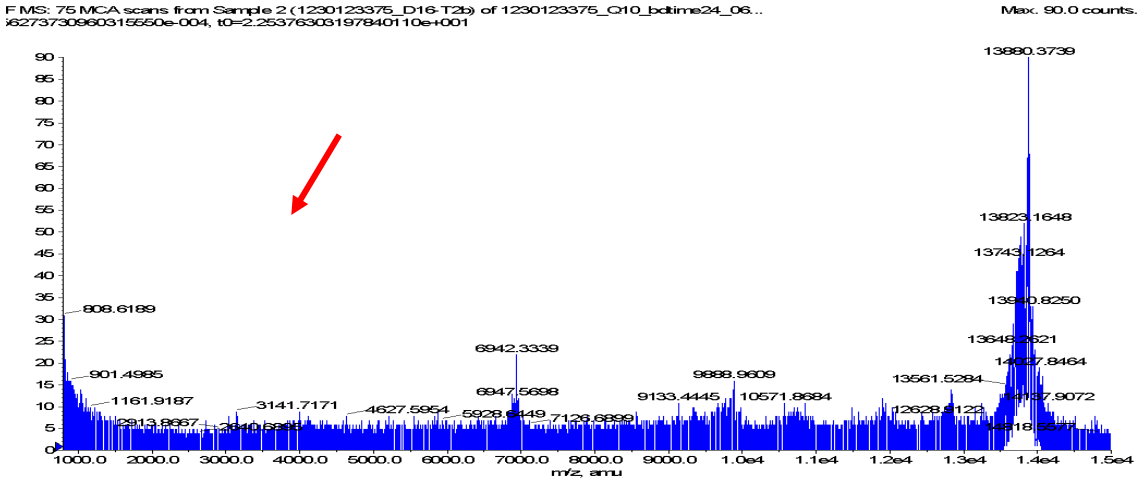
Biospecimen Procurement Recommendations: **Fresh frozen tissue**
Rapid procurement

Courtesy K. Calvo NCI

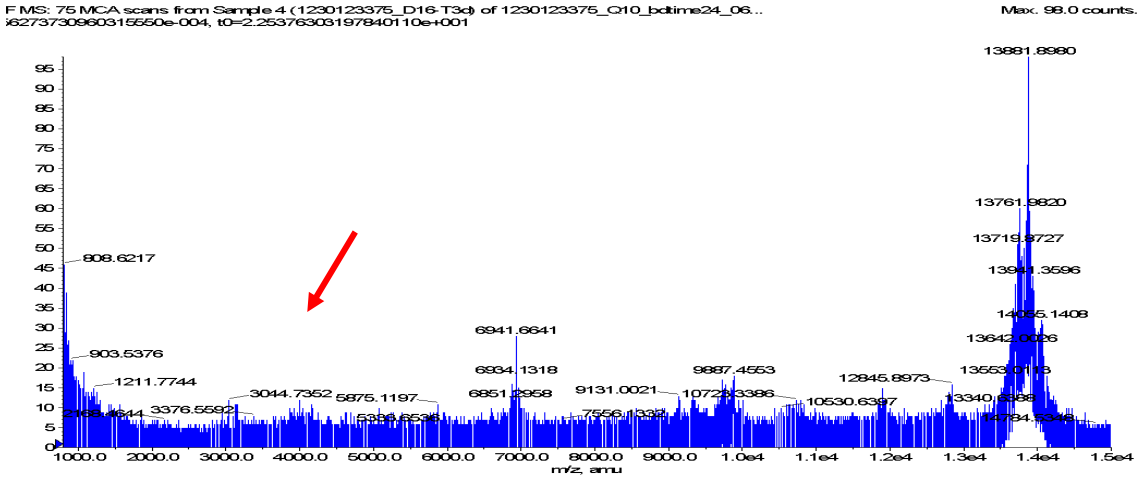
Protein instability in Mass Spect analyses

Comparing Plasma Spectra: Same donor at 24 Hrs

-Notice the "missing" peaks in raw plasma



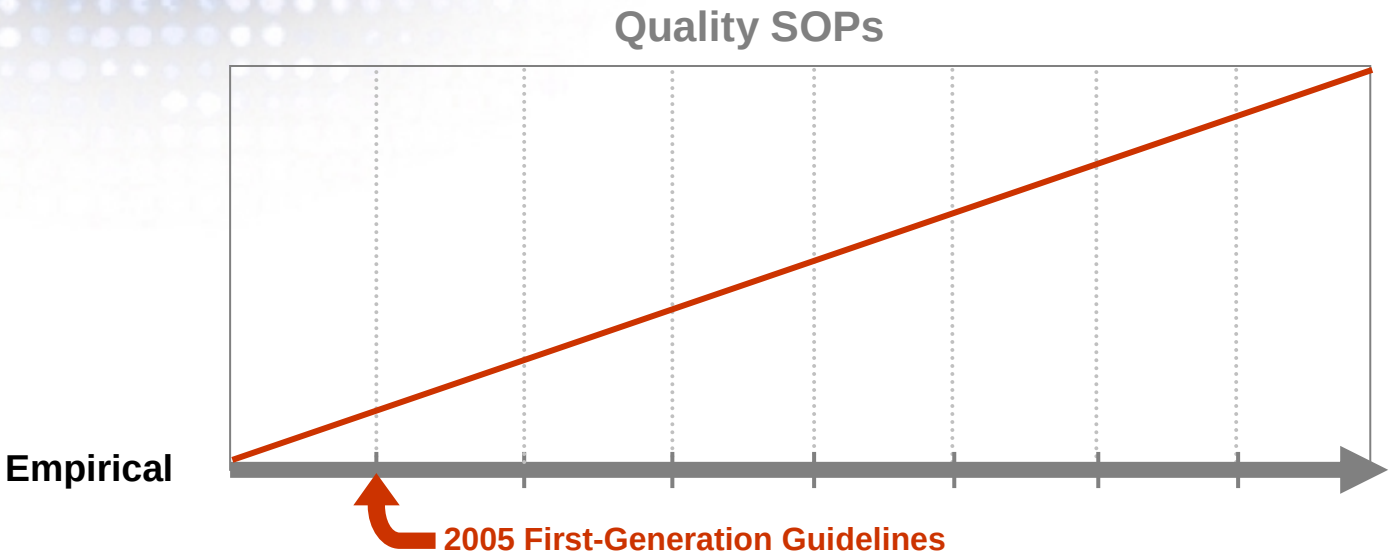
Raw Plasma



Plasma in Protease Inhibitor

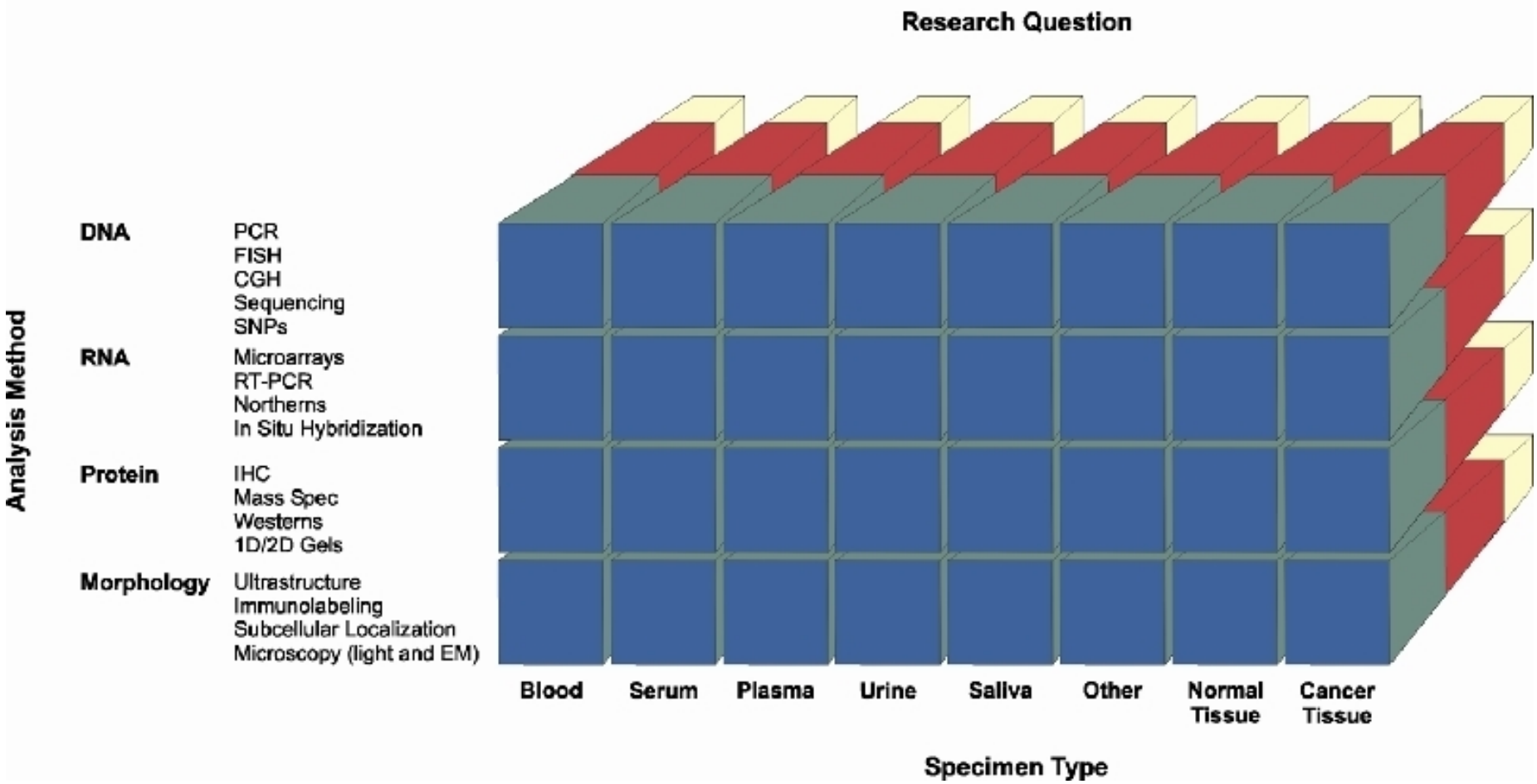
Courtesy G. Whiteley – NCI Clinical Proteomics Program

The Future: Pathway to Scientifically Validated Biorepository Practices



Medical/ Surgical Procedures	Acquisition	Handling/ Processing	Storage	Distribution	QC/QA	Restocking Unused Sample

Suggested Framework for Future Development of 2nd Generation Research-Based Best Practices



How the NCI OBBR will help

- Publish 1st Generation Guidelines and provide information to biorepositories to assist in understanding and implementing them.
- Establish a biospecimen research network. Some of the work from this proteomics program will be very relevant.
- Use data from the network to develop the next generation of evidence-based guidelines.
- Provide a forum for exchange of information about biospecimen issues.
- Provide educational resources to the biorepository community.

OBBR web site – http://biospecimens.cancer.gov

Email – biospecimens@mail.nih.gov